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**A novel method for sex estimation using 3D computed tomography models of tooth roots:
a volumetric analysis**

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Introduction

Estimating the sex of an unknown individual is an important step in the identification of human skeletal remains. Teeth, as one of the strongest tissues in the human body, can be used for this purpose. Most studies on dental sexual dimorphism are based on crown and cervical mesiodistal and buccolingual diameters of teeth (Acharya & Mainali, 2007; Hassett, 2011; Viciano, Lopez-Lazaro, & Aleman, 2013; Kazzazi & Kranioti, 2016). These studies have shown that the canine is the most sexually dimorphic tooth, providing accuracy rates in this respect of over 85% (Hassett, 2011, Viciano, D'Anastasio, & Capasso, 2015; Kazzazi & Kranioti, 2016). Other studies have shown that the length of root measurements are highly sexually dimorphic and therefore can be used for sex estimation (Garn, Van Alstine, & Cole, 1978; Harris & Couch, 2006; Zorba, Vanna, & Moraitis, 2014). In addition, root measurements are not affected by wear, as is often the case with crown measurements (Hillson, FitzGerald, & Flinn, 2005). Lähdesmäki and Alvesalo (2004; 2007) report that the influence of Y chromosomes on the development of root length is greater than of the X chromosome, which may be the cause of sexual dimorphism in root size. It is probable, therefore, that tooth root volume is different between males and females.

During the last decade, advances in imaging techniques such as computed tomography (CT) have enabled researchers to create highly accurate volume rendering models and multiplanar reconstructions. This allows for a better examination of sexually dimorphic characteristics of the tooth, especially the root, which is usually hidden in the jaw. There are many studies validating the efficacy of CT scans as a tool to measure both root length and volume (Liu et al., 2010; Lund, Grondahl, Hansen, & Grondahl, 2012; Kim, Lim, Gang, & Kim, 2013; Forst et al., 2014). Liu et al. (2010) conducted in vivo volumetric determination using CT scan images and demonstrated small differences (within -4% to +7%) from the actual physical volumes. However, tooth volume has rarely been used for sex estimation in archaeological or forensic studies. In two studies by Tardivo et al. (2011; 2015) the pulp volume and the total volume of the maxillary and mandibular canines were used for sex estimation. The authors reported that canine volume measurements are highly sexually dimorphic and provided an accuracy classification rate of 100%. However, the validity of this method in sex estimation needs to be tested using other tooth types. In addition, dental pathologies such as caries (particularly occlusal carries) and tooth wear will considerably affect the size of the tooth crown, and in more severe cases will result in pulp exposure (Scully, Flint, & Stephen, 2010; Van Noort, 2013)

and eventually alter the measurements. The current study attempts to overcome these issues by measuring the volume of the root from the apical to the cement-enamel junction (CEJ).

It has long been acknowledged that metric sex estimation methods are population-specific because of variation in size and sexual dimorphism patterns. Currently, there are very few studies of sex prediction using odontometric data in either Iranian archaeological or modern populations. The only previous study was done by Kazzazi and Kranioti (2016) using cervical mesiodistal and buccolingual measurements for sex estimation in the same population. Following up on the previous work, the present study makes a significant contribution to the development of the standards of sex assessment. In addition to this, the unusual way in which the sample of the Hasanlu population – the focus of the current study – was formed means there is distinctive anthropological and archaeological value to the population sample. The majority of the biological remains are from the victims of an instantaneous catastrophe, which results in synchronicity in the paleodemographic data. Moreover, the Hasanlu osteological collection represents one of only a few well-preserved skeletal collections from Iron Age Iran.

The aim of the present study is two-fold: a) to examine the level of sexual dimorphism in the permanent teeth of Iranian archaeological populations using the volume of tooth roots, and b) to provide an easy and precise technique for sex estimation using 3D volumetric models of tooth roots that can be used in both forensic and archaeological sets of dentition.

Material and Method

Material

The study was carried out on the skeletal remains of 52 individuals from two of the most significant archaeological sites in north-western Iran. The individuals come from two sites in the Gadar River Valley in West Azerbaijan: 28 from Hasanlu, dating from 1450 to 800 BC, and 24 from Dinkha Tepe (15 miles west of the Hasanlu site), dating from 1350 to 800 BC. The Hasanlu settlement was destroyed by fire around 800 BC, after a battle, most likely with Urartians (Dyson, 1965). The two sites are of similar age (Muscarella, 1988). The main focus of this study are the Dinka II and III burials (1350-800 BC) and the Hasanlu periods IV and V (1450-800 BC), which contain analogous material (Muscarella, 1974; 1988). The remains from the Hasanlu and Dinkha Tepe sites are now stored in the University of Pennsylvania's Museum of Archaeology and Anthropology (UPM).

The total number of teeth analysed in this study was 478, consisting of maxillary and mandibular teeth from 52 skeletons (32 males, 20 females). These 52 individuals were all adults

aged between 20 and 65 years; their age at death was estimated based on amount of dental wear (Miles, 2001; Buikstra & Ubelaker, 1994), changes in the pubic symphyseal face (Brooks & Suchey, 1990) and ilium auricular area (Buckberry & Chamberlain, 2002), and closure of cranial sutures (Meindl & Lovejoy, 1985). The sex of each skeleton was estimated using morphological features of the pelvis (ventral arch, subpubic concavity, and ischiopubic ramus (Phenice, 1969)) and skull (nuchal crest, mastoid process, glabella, supraorbital margin, mental eminence, and shape of orbit (Walker, 2008)).

Scanning protocol

In total 52 skulls were scanned at the Hospital of the University of Pennsylvania using a Siemens Somatom sensation 64-slice Computed Tomography machine. Data were collected using a slice thickness of 0.5 mm and a matrix of 512×512 pixels. All data were saved in the Digital Imaging and Communications in Medicine (DICOM) format.

The CT scans used in this study were obtained through the Open Research Scan Archive (ORSA).

Data acquisition

3D models of the teeth were created using manual segmentation in the Amira 6.01 software package. Since tooth density largely differs from crown to apex, root segmentation requires more than one threshold level. In this study the threshold level was adjusted two times: first to segment the root from the jaw and second to segment the crown from the root. Thresholds used for root segmentation were calculated using the half maximum height protocol of Spoor, Zonneveld, & Macho (1993) for each skull, and thresholds used for crown segmentation were set visually for each tooth separately. The segmentation protocol used in Amira was performed on consecutive 2D slices, and the magic wand, as a region growing tool, was used to select the appropriate tooth portion for each slice. Segmentation was mainly processed in the axial view from crown to apex. Crown and root of the same tooth were colour coded to facilitate differentiation (Figure 1). No smoothing functions were applied to the 3D tooth structure. Liu et al. (2010) reported that the use of the smoothing function caused a reduction of the tooth root volume measurement by about 3-12%. Once segmentation was complete, the software automatically computed the volume of the root.

Volume measurements were taken on loose teeth as well as on teeth intact in the jaw. Dental root volume was taken from the cement-enamel junction (CEJ) to the apex of the tooth, including the pulp chamber and canals.

Root volume measurements were taken from the right maxillary and mandibular teeth. To avoid the possibility of incorrect measurements, the samples with root resorption, incomplete root formation, a broken root, root caries, or caries along the CEJ were excluded. Measurements of maxillary third molars were excluded from the discriminant function analysis, due to the small number of these teeth in the sample, and their wide variation in size (Townsend, Brook, Yong, & Hughes, 2016). In total 464 teeth were used for sex estimation.

Statistical analysis

Inter- and intra-observer error were estimated using, respectively, 30 and 35 randomly selected individuals from the original sample. Technical error of measurements (TEM), relative technical error of measurements (rTEM), and the coefficient of reliability R (Ulijaszek & Kerr, 1999) were used to determine the differences between measurements.

Normality was evaluated using the Kolmogorov-Smirnov test and Levene's test was run to evaluate homogeneity of variances. A one-way ANOVA was used to compare the mean differences between males and females. In order to determine whether there were any statistically significant differences between the Hasanlu and Dinkha Tepe collections, an independent sample t-test was conducted. Garn, Lewis, Swindler, & Kerewsky's formula (1967) was used to calculate the percentage of sexual dimorphism for the volume of the root measurement: $[(\text{male mean} - \text{female mean}) / \text{female mean}] \times 100$. The percentage of sexual dimorphism shows the difference between male and female mean values. A positive result indicates larger tooth size in males and a negative result indicates larger tooth size in females.

Univariate discriminant function analysis was used for each variable. Stepwise discriminant function analysis was applied in order to determine which variables best distinguished between males and females. Root volume measurements were separated by tooth class (incisor, canine, premolar, and molar) and position (maxillary and mandibular), and discriminant analysis was conducted separately for each measurement. Previous studies on crown and cervical mesiodistal and buccolingual measurements have demonstrated that canines are the most sexually dimorphic teeth, therefore we added maxillary canines to each function to determine whether classification accuracy would increase. Discriminant function analysis was only carried out for variables which showed a sample size greater than 20.

Bootstrapping was used in all cases to account for possible biases due to the small sample size.

In order to demonstrate the accuracy rate of the original sample and the sample created by cross-validation, the study applied a leave-one-out classification procedure. Posterior

probabilities for each individual were calculated; these reflect the suitability of each case to be reassigned to the original group according its discriminant score (Kranioti, Işcan, & Michalodimitrakakis, 2008). Statistical analysis was carried out using the SPSS 23 software.

Results

Four hundred and sixty four three-dimensional tooth models were constructed from computed tomography (CT) scans from the Hasanlu and Dinkha Tepe skeletons. Intra- and inter-observer TEM, rTEM, and the coefficient of reliability (R) for the tooth volume measurements are provided in Table 1. Intra-observer error is low and inter-observer error is slightly higher. The mean rTEM for intra- and inter-observer error was 1.54% and 1.75% respectively, with a coefficient variation of > 0.98 for all measurements. The variables with the highest value for inter- and intra-observer error were the mandibular fourth premolar (2.06%) and maxillary second incisor (2.27%) respectively.

The Kolmogorov-Smirnov test showed that the distribution of all variables was normal. The results of the homogeneity of variance test also indicated that the sample was statistically homogenous for all variables. The results of the independent student *t*-test showed that there were no statistically significant differences between the mean values of Hasanlu and Dinkha Tepe skeletons ($p < 0.05$) (Table 2). Accordingly, the two samples were subsequently pooled to increase sample size for analysis.

The one way ANOVA indicated that the differences between male and female mean values were significant for all measurements ($p < 0.001$). Table 3 shows descriptive statistics, associated univariate *F*-ratio, and percentage of sexual dimorphism. The *F*-ratio indicates the degree of variation within and between the sexes, as well as the significance level of the variance (Işcan & Shihai, 1995). The mean values of the male measurements were significantly higher than those of the females. The most sexually dimorphic tooth was the maxillary second incisor, with a 106.33 percentage of dimorphism, followed by the maxillary canine and maxillary first incisor, each with a 96.43 and 75.77 percentage of dimorphism respectively (Table 3). It should be noted that, due to the small sample size, the results presented in Table 3 should be taken as an illustration of the sex differences in the tooth root volume measurements in the study sample, and not a representative of differences in the population.

Discriminant function analysis was carried out for samples of > 20 individuals that had relatively equal male/female size groups. The maxillary M3 was removed from the discriminant analysis due to the small number of data. Table 4 shows the coefficient indicating

the relative contribution of each dimension, degree of freedom (df) and the F values for stepwise discriminant functions. Function 1 to 7 demonstrate the results of discriminant analysis using the root volumes of each tooth type. The maxillary canine was added to functions 8 to 12 to examine whether classification accuracy would increase. Once the variables that produced the maximum discrimination were obtained, univariate discriminant analysis was used to produce various other functions. Table 5 shows the coefficient, degree of freedom, and F values for each tooth separately. The sectioning point was set to zero for all functions, meaning that if the value obtained was greater than zero, the individual was considered male, and if less than zero the individual was considered female.

Table 6 shows the classification accuracy of all functions. All of the stepwise discriminant functions provided classification accuracy of over 90%, with an accuracy rate of 100% for half of the functions (functions 2, 3, 8, 10, 11, 12). The combination of maxillary third and fourth premolars with the maxillary canine (function 9) gave the next best classification (96.6%), followed by the mandibular first and second incisors (function 1: 96.3%). By adding the maxillary canine to functions 8 to 12, classification accuracy was significantly improved and all of the functions provided an accuracy rate of 100%, except function 9 (96.6%) (Table 6). The best single variable was found to be the maxillary second incisor (100%), followed by the maxillary canine (97.1%) and the mandibular second incisor (96.6%). In most functions accuracy in males was greater than in females. Accuracy ranged from 86.7-100% in males and 63.5-100% in females (Table 6). Cross validation accuracy was close to the original classification accuracy in all cases (Table 6).

Figures 2 and 3 shows the probability levels of correct group assessment according to the discriminant scores of each individual. For example, if a discriminant score based on the stepwise analysis of the root volume measurement of the mandibular first incisor (function 1) is 3.39 (x coordinate), the posterior probability of that individual coming from the male group is 100% (y coordinate).

Discussion

Computed tomography (CT) is a non-invasive technique that allows for an accurate and detailed visualization of morphological features without causing any tooth destruction (Bergmans, Van Cleynenbreugel, Wevers, & Lambrechts, 2001). Such scans can yield a substantial

amount of information, due to the ability of the slices to be recreated in any plane and the data to be represented in the form of 2D and 3D images. The simultaneous or separate demonstration of internal and external anatomy is also possible, as well as qualitative and quantitative assessment of the images (Rhodes, Pitt Ford, Lynch, Liepins, & Curtis, 1999). These features, along with the ability of CT to provide reproducible data in all three dimensions, have resulted in an increase in the usage of this method in anthropological and dental research.

Odontometric sex estimation methods are considered to be population specific, therefore many studies have developed odontometric standards for different populations based on different dental measurements (Harris & Couch, 2006; Acharya & Mainali, 2007; Hassett, 2011; Viciano, Lopez-Lazaro, & Aleman et al. 2013). Unfortunately, very few population-specific odontometric standards have been developed for Iranian populations. The only example is a recent study by Kazzazi and Kranioti (2016) on cervical mesiodistal and buccolingual measurements in Hasanlu and Dinkha Tepe populations. In the present study, the CT scan images of the same populations were used to examine the validity of tooth root volume measurements in sex estimation.

Tooth root volume measurements can be especially effective in the case of samples containing poorly preserved bones and highly worn teeth. Since the sample size in this study is relatively small, the data provided here could possibly be used as a baseline for other comparisons. However, we demonstrate the significant potential of tooth root volume measurements for sex assessment in archaeological samples. This can potentially be applied in forensic scenarios if appropriate reference samples of modern populations are put in test.

In the present study, the quality of the CT images of the mandible was better than that of the maxillae. This might be because of a bigger contrast between the dental alveolus and the cortex that surrounds it, resulting in a better visualization. Nevertheless, during the course of the research, no differences between the segmentation processes of upper and lower teeth were detected. It was noticed that the density of the root in both the upper and lower jaws was closer to cortical bone and easily visualized. A problematic situation occurred when the teeth root were adjacent to cortical bone in the mandible, making segmentation relatively more difficult.

Precision testing demonstrated low intra-observer error with R values > 0.99 , $rTEM < 2.06\%$, and $TEM < 4.65$. These results are slightly higher than in the previous study on cervical mesiodistal and buccolingual measurements in Hasanlu and Dinkha Tepe skeletons. However, the TEM values were significantly higher (< 4.65) compared with the cervical measurements (< 0.07) (Kazzazi & Kranioti, 2016). This difference is due to the positive association between the size of the TEM and the size of the measurements. For example, a large mean value will

have a large TEM, and thus the comparison of measurements of different size cannot be assessed (Ulijaszek & Kerr, 1999). To overcome this problem, the TEM can be transferred to relative TEM (rTEM%), which is the error expressed as a percentage that corresponds to the total average of the variable analysed. The rTEM value for tooth root volume measurements (< 2.06) was slightly higher than that for cervical measurements (< 1.81). The results of inter-observer error analysis also showed R values of < 0.98 and rTEM of $< 2.27\%$.

This study demonstrated significant sexual dimorphism, and mean values were considerably higher in males compared with females. A comparison between the two sexes showed that the classification accuracy of most functions was higher for males. This means that females have a greater variation in tooth size and can more often be misclassified as male. This result is in agreement with the previous study (Kazzazi & Kranioti, 2016) on cervical mesiodistal and buccolingual measurements of Hasanlu and Dinkha Tepe skeletons.

The percentages of sexual dimorphism were also calculated for the tooth root volume measurements. Unfortunately, sample size for most variables is too small to make a significant comparison. Moreover, there is no comparative data against which the degree of sexual dimorphism in tooth root volume can be compared. However, a provisional comparison with crown, cervical, and root measurements can be performed, which describes the patterns in the sample and which might underline some interesting similarities or differences.

In the previous study of cervical measurements of Hasanlu and Dinkha Tepe skeletons the greatest difference in percentage of sexual dimorphism was observed in the mandibular canine (Kazzazi & Kranioti, 2016), however analysis of root volume showed the highest percentage of sexual dimorphism in the maxillary second incisor. This result is in accordance with the results of Tuttösi and Cardoso's (2015) study of tooth cervical measurements and Zorba, Vanna, and Moraitis's (2014) study of root length measurements. The maxillary canine was the next tooth showing the highest percentage of sexual dimorphism; this tooth has also been reported high for sexual dimorphism in crown, cervical, and root length measurements in the literature (Garn, Cole, & Van Alstine, 1979; Cardoso, 2008; Zorba, Vanna, & Moraitis, 2014). These teeth also provided the highest classification accuracy rate for the univariate discriminant function analysis. For the molar teeth, the second molar showed the highest percentage of sexual dimorphism in accordance with the results of Tuttösi and Cardoso (2015) and also those of crown measurement studies (Cardoso, 2008; Garn, Cole, & Van Alstine, 1979; Zorba, Moraitis, & Manolis, 2011).

Univariate discriminant function analysis also showed that the root volume of incisors and canines was the most dimorphic, providing classification accuracy between 89.3-100%. These

results were in accordance with Kazzazi and Kranioti (2016) and other studies on crown and cervical measurements (Hassett, 2011; Viciano, Lopez-Lazaro, & Aleman, 2013; Viciano, D'Anastasio, & Capasso, 2015). The root volume of the maxillary second incisor reached the highest accuracy rate (100%), followed by the maxillary canine, which correctly classified sex in 97.10% of the sample. This result is in accordance with the study by Tardivo et al. (2015) of sexual dimorphism in the total volume of canines. However, Kazzazi and Kranioti (2016) and most previous studies on crown and cervical measurements (Saunders, Chan, Kahlon, Kluge, & FitzGerald, 2007; Angadi, Hemani, Prabhu, & Acharya, 2013; Khamis, Taylor, Malik, & Townsend, 2014; Viciano, D'Anastasio, & Capasso, 2015) have demonstrated a greater sexual dimorphism in the dimensions of mandibular canines. The root volume of the maxillary second molar also provided the highest accuracy rate among molar teeth (90.3%). Similar results were achieved by Kazzazi and Kranioti (2016) and other studies on cervical and crown measurements (Zorba, Moraitis, Eliopoulos, & Spiliopoulou, 2012; Viciano, D'Anastasio, & Capasso, 2015; Tuttösí & Cardoso, 2015). In addition, this study showed that the root volume of premolar teeth can provide very effective variables for sex estimation, with a classification accuracy ranged between 84.4% and 90.9%. However, the analysis of Hasanlu and Dinkha Tepe dental cervical measurements reported a low accuracy rate of 75.9-79.3% for premolars (Kazzazi & Kranioti, 2016). This short comparison should be taken cautiously, due to the sample size and the comparability of root volume, crown and cervical measurements. Although it recognised that there are some possible similarities and differences in sex differences among these measurements.

Furthermore, several multivariate discriminant functions were created using different combinations of variables. The results of the stepwise discriminant function analysis indicated that the prediction accuracies for estimating sex using the volume of the tooth root measurements ranged from 90 to 100% in Hasanlu and Dinkha Tepe skeletons, with accuracy in males higher than in females. 6 out of the 12 functions used in the discriminant function analysis provided an accuracy rate of 100% for both original and cross-validate data. In general, the classification accuracy rate for both univariate and stepwise discriminant function analyses was better than those reported for cervical measurements of Hasanlu and Dinkha Tepe skeletons (Kazzazi & Kranioti, 2016), showing that the volume of the tooth root can also be useful for sex estimation, however the results must be interpreted with caution due to the small sample size.

The novel sex estimation technique proposed here represents an easy and simple technique based on tooth root volume measurements taken on CT images of permanent teeth. Based on

the set of data used here, the estimation of sex using tooth root volume measurements is successful. Inter- and intra-observer error was low for all variables. Therefore it appears that the technique proposed in this study is a valid alternative for sex estimation with the added value that no manual handling of the teeth –which may accidental destruction-, is necessary. Although the present study is based on the analysis of archaeological samples, the advantages of this new method make it an affective and useful technique in forensic human identification.

The increasing use of medical imaging modalities in the forensic investigation of death in the last decades (Knight, 1984; Evans & Knight, 1986; Kahana & Hiss, 1997; Brogdon, 2010) has proven that these are extremely useful in cases of decomposed and mutilated or fragmented remains-a common issue in forensic settings. Similar to archaeological analysis, one of the extra advantages of computed-generated anatomical models is giving the forensic anthropologists the opportunity to study the fragile “real specimens” without having to handle them. In most forensic cases, the remaining soft tissue is attached to dental and skeletal specimens that are hard to remove or deflesh. Due to this soft tissue, significant features and landmarks that can be used in determining the biological profile could be obscured. Virtual 3D models allow the forensic anthropologists to virtually remove the soft tissue or to make it transparent for the analysis.

Virtual models of teeth and bones allow for partial remains to be duplicated or to create virtual casts of the space that once was occupied by an anatomical structure in order to continue with analysis. An excellent example of this advantageous technology is the development of a sex estimation method based on the bony labyrinth (Osipov et al., 2013).

It must be stressed, however, that this study used an osteologically estimated sex sample to test the method. Despite the high level of accuracy of morphological techniques of sex assessment, the true sex of each of these individuals is unknown. It is therefore likely that the percentage of correct classifications of known sex may be a small degree higher or lower than in the presented data (Tuttösi & Cardoso, 2015). The other major limitation of this study was its small sample size. The study must be expanded, therefore, with a larger data sample to refine the proposed new method. However, the importance of this study lies in the fact that it can be applied to unknown skeletal remains belonging to the same period (the Iron Age) in Iran. This becomes even more important considering that teeth have better chances of survival in harsh taphonomic conditions compared with other skeletal elements. It is highly recommended that the reliable estimates be considered, with over 95% probability of correct classification. However, with regard to estimates with 80-95% probability, the predictions should be treated

with caution, and when the probability rate of the estimate is lower than 80%, the method should be considered unreliable and other methods should be used instead.

Following the positive results of this study the method should be applied in large contemporary samples in order to create forensic standards for sex estimation for different populations. Unpublished data from a pilot study conducted by the authors in a sample of twenty modern individuals from the Cretan collection (Kranioti, İşcan, & Michalodimitrakis, 2008; Kranioti & Michalodimitrakis, 2009) resulted in similarly high classification accuracy for molars which reinforces the belief that this method can be extremely valid in forensic applications. For such purpose though, following the existing evidentiary rules and the recommendations of the professional associations for the admissibility of scientific evidence, the reliability and accuracy must be calculated and error rates must be reported before any new method can be put in use.

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